PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: **Mikal E. Saltveit** *et al.*

Serial No.: **09/964,992**

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For: **Characterization of Phenylalanine Ammonia-lyase (PAL) Gene in Wounded Lettuce Tissue**

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Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The following is the replacement page 5 including the amendment to lines 1-2 shown on the attached "Version with Markings to Show Changes Made" and a formal drawing of figure 9 including the amendment shown on the attached "Red Sketch of Drawing to Show Changes Made".

CERTIFICATE OF FIRST CLASS MAILING

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Respectfully submitted,

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Figure 9: Sequence comparison of LsPAL1 (SEQ ID NO:1) to sunflower PAL (SEQ ID NO:5) demonstrating the close sequence homology.

Figure 10: Semi-quantitative LsPAL1 RNA expression demonstrating the temporal regulation of PAL1 in response to wounding. RNA levels peak at 12 hours post-wounding and decline to near baseline levels at 36 hours post-wounding.

Figure 11: Identification of wounding products from a 1cm piece of lettuce midrib, tested in three equal length segments 12 and 24 hours post-wounding. A) Cinnamic acid concentration at 12 and 24 hours compared to control lettuce segments. Cinnamic acid is the second product produced in the phenylpropanoid pathway as depicted in figure 1. B) LsPAL1 expression 12 hours post-wounding compared to control lettuce segments.

Figure 12: Distribution of LsPAL1 RNA in epidermal, vascular and cortex lettuce tissue in response to wounding.

Detailed Description of the Invention

In accordance with the subject invention, nucleic acid and protein sequences obtainable from a plant source are provided which are capable of catalyzing the formation of trans-cinnamic acid by the deamination of L- phenylalanine. Such proteins are referred to herein as phenylalanine ammonia-lyase proteins, or PAL.

Numerous reports in the literature detail how the activity of the phenylpropanoid pathway in plants is increased following abiotic and biotic stresses. Increased activity of this pathway results in the synthesis and accumulation of phenolic compounds that contribute to would healing, plant defense and tissue browning. The first committed enzyme in this pathway is phenylalanine ammonia-lyase (PAL), which also controls the rate at which phenolic compounds are produced by this and subsequent pathways. Possession of the gene allows its manipulation by genetic engineering techniques to enhance or suppress its action. Tissue can now be produced with enhanced disease resistance, or demonstrating suppressed browning potential following wounding.

The peptide sequences provided are useful for obtaining polynucleotide sequences which encode PAL, and sequences associated with the expression of PAL in response to wounding. The obtained nucleic acid sequences find use in the preparation of constructs to direct their expression in a host cell. The sequences also provide means for adopting strategies to use physical or chemical methods to inactivate or disrupt the PAL activity, or expression of the PAL protein.